TITLE OF THE INVENTION

PROTEIN-PROTEIN INTERACTIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application is related to U.S. provisional patent application Serial No. 60/256,983, filed on 21 December 2000, incorporated herein by reference, and claims priority thereto under 35 USC §119(e).

BACKGROUND OF THE INVENTION

[0002] The present invention relates to the discovery of novel protein-protein interactions that are involved in mammalian physiological pathways, including physiological disorders or diseases. Examples of physiological disorders and diseases include non-insulin dependent diabetes mellitus (NIDDM), neurodegenerative disorders, such as Alzheimer's Disease (AD), and the like. Thus, the present invention is directed to complexes of these proteins and/or their fragments, antibodies to the complexes, diagnosis of physiological generative disorders (including diagnosis of a predisposition to and diagnosis of the existence of the disorder), drug screening for agents which modulate the interaction of proteins described herein, and identification of additional proteins in the pathway common to the proteins described herein.

[0003] The publications and other materials used herein to illuminate the background of the invention, and in particular, cases to provide additional details respecting the practice, are incorporated herein by reference, and for convenience, are referenced by author and date in the following text and respectively grouped in the appended Bibliography.

[0004] Many processes in biology, including transcription, translation and metabolic or signal transduction pathways, are mediated by non-covalently associated protein complexes. The formation of protein-protein complexes or protein-DNA complexes produce the most efficient chemical machinery. Much of modern biological research is concerned with identifying proteins involved in cellular processes, determining their functions, and how, when and where they interact with other proteins involved in specific pathways. Further, with rapid advances in genome sequencing, there is a need to define protein linkage maps, i.e., detailed inventories of protein interactions that make up functional assemblies of proteins or protein complexes or that make up physiological pathways.

[0005] Recent advances in human genomics research has led to rapid progress in the identification of novel genes. In applications to biological and pharmaceutical research, there is a

need to determine functions of gene products. A first step in defining the function of a novel gene is to determine its interactions with other gene products in appropriate context. That is, since proteins make specific interactions with other proteins or other biopolymers as part of functional assemblies or physiological pathways, an appropriate way to examine function of a gene is to determine its physical relationship with other genes. Several systems exist for identifying protein interactions and hence relationships between genes.

[0006] There continues to be a need in the art for the discovery of additional protein-protein interactions involved in mammalian physiological pathways. There continues to be a need in the art also to identify the protein-protein interactions that are involved in mammalian physiological disorders and diseases, and to thus identify drug targets.

SUMMARY OF THE INVENTION

[0007] The present invention relates to the discovery of protein-protein interactions that are involved in mammalian physiological pathways, including physiological disorders or diseases, and to the use of this discovery. The identification of the interacting proteins described herein provide new targets for the identification of useful pharmaceuticals, new targets for diagnostic tools in the identification of individuals at risk, sequences for production of transformed cell lines, cellular models and animal models, and new bases for therapeutic intervention in such physiological pathways

[0008] Thus, one aspect of the present invention is protein complexes. The protein complexes are a complex of (a) two interacting proteins, (b) a first interacting protein and a fragment of a second interacting protein, (c) a fragment of a first interacting protein and a second interacting protein, or (d) a fragment of a first interacting protein and a fragment of a second interacting protein. The fragments of the interacting proteins include those parts of the proteins, which interact to form a complex. This aspect of the invention includes the detection of protein interactions and the production of proteins by recombinant techniques. The latter embodiment also includes cloned sequences, vectors, transfected or transformed host cells and transgenic animals.

[0009] A second aspect of the present invention is an antibody that is immunoreactive with the above complex. The antibody may be a polyclonal antibody or a monoclonal antibody. While the antibody is immunoreactive with the complex, it is not immunoreactive with the component parts of the complex. That is, the antibody is not immunoreactive with a first interactive protein, a fragment of a first interacting protein, a second interacting protein or a fragment of a second

interacting protein. Such antibodies can be used to detect the presence or absence of the protein complexes.

[0010] A third aspect of the present invention is a method for diagnosing a predisposition for physiological disorders or diseases in a human or other animal. The diagnosis of such disorders includes a diagnosis of a predisposition to the disorders and a diagnosis for the existence of the disorders. In accordance with this method, the ability of a first interacting protein or fragment thereof to form a complex with a second interacting protein or a fragment thereof is assayed, or the genes encoding interacting proteins are screened for mutations in interacting portions of the protein molecules. The inability of a first interacting protein or fragment thereof to form a complex, or the presence of mutations in a gene within the interacting domain, is indicative of a predisposition to, or existence of a disorder. In accordance with one embodiment of the invention, the ability to form a complex is assayed in a two-hybrid assay. In a first aspect of this embodiment, the ability to form a complex is assayed by a yeast two-hybrid assay. In a second aspect, the ability to form a complex is assayed by a mammalian two-hybrid assay. In a second embodiment, the ability to form a complex is assayed by measuring *in vitro* a complex formed by combining said first protein and said second protein. In one aspect the proteins are isolated from a human or other animal. In a third embodiment, the ability to form a complex is assayed by measuring the binding of an antibody, which is specific for the complex. In a fourth embodiment, the ability to form a complex is assayed by measuring the binding of an antibody that is specific for the complex with a tissue extract from a human or other animal. In a fifth embodiment, coding sequences of the interacting proteins described herein are screened for mutations.

[0011] A fourth aspect of the present invention is a method for screening for drug candidates which are capable of modulating the interaction of a first interacting protein and a second interacting protein. In this method, the amount of the complex formed in the presence of a drug is compared with the amount of the complex formed in the absence of the drug. If the amount of complex formed in the presence of the drug is greater than or less than the amount of complex formed in the absence of the drug, the drug is a candidate for modulating the interaction of the first and second interacting proteins. The drug promotes the interaction if the complex formed in the presence of the drug is greater and inhibits (or disrupts) the interaction if the complex formed in the presence of the drug is less. The drug may affect the interaction directly, i.e., by modulating the binding of the two proteins, or indirectly, e.g., by modulating the expression of one or both of the proteins.

[0012] A fifth aspect of the present invention is a model for such physiological pathways, disorders or diseases. The model may be a cellular model or an animal model, as further described herein. In accordance with one embodiment of the invention, an animal model is prepared by creating transgenic or "knock-out" animals. The knock-out may be a total knock-out, i.e., the desired gene is deleted, or a conditional knock-out, i.e., the gene is active until it is knocked out at a determined time. In a second embodiment, a cell line is derived from such animals for use as a model. In a third embodiment, an animal model is prepared in which the biological activity of a protein complex of the present invention has been altered. In one aspect, the biological activity is altered by disrupting the formation of the protein complex, such as by the binding of an antibody or small molecule to one of the proteins which prevents the formation of the protein complex. In a second aspect, the biological activity of a protein complex is altered by disrupting the action of the complex, such as by the binding of an antibody or small molecule to the protein complex which interferes with the action of the protein complex as described herein. In a fourth embodiment, a cell model is prepared by altering the genome of the cells in a cell line. In one aspect, the genome of the cells is modified to produce at least one protein complex described herein. In a second aspect, the genome of the cells is modified to eliminate at least one protein of the protein complexes described herein.

[0013] A sixth aspect of the present invention are nucleic acids coding for novel proteins discovered in accordance with the present invention and the corresponding proteins and antibodies.

[0014] A seventh aspect of the present invention is a method of screening for drug candidates useful for treating a physiological disorder. In this embodiment, drugs are screened on the basis of the association of a protein with a particular physiological disorder. This association is established in accordance with the present invention by identifying a relationship of the protein with a particular physiological disorder. The drugs are screened by comparing the activity of the protein in the presence and absence of the drug. If a difference in activity is found, then the drug is a drug candidate for the physiological disorder. The activity of the protein can be assayed *in vitro* or *in vivo* using conventional techniques, including transgenic animals and cell lines of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

[0015] The present invention is the discovery of novel interactions between proteins described herein. The genes coding for some of these proteins may have been cloned previously,

but their potential interaction in a physiological pathway or with a particular protein was unknown. Alternatively, the genes coding for some of these proteins have not been cloned previously and represent novel genes. These proteins are identified using the yeast two-hybrid method and searching a human total brain library, as more fully described below.

[0016] According to the present invention, new protein-protein interactions have been discovered. The discovery of these interactions has identified several protein complexes for each protein-protein interaction. The protein complexes for these interactions are set forth below in Tables 1-10, which also identifies the new protein-protein interactions of the present invention.

TABLE 1

Protein Complexes LXR-alpha/Utrophin Interaction

Oxysterol liver X receptor alpha (LXR-alpha) and utrophin

A fragment of LXR-alpha and utrophin

LXR-alpha and a fragment of utrophin

A fragment of LXR-alpha and a fragment of utrophin

TABLE 2

Protein Complexes LXR-alpha/zyxin Interaction

Oxysterol liver X receptor alpha (LXR-alpha) and zyxin

A fragment of LXR-alpha and zyxin

LXR-alpha and a fragment of zyxin

A fragment of LXR-alpha and a fragment of zyxin

TABLE 3

Protein Complexes LXR-alpha/LIMS1 Interaction

Oxysterol liver X receptor alpha (LXR-alpha) and LIMS1

A fragment of LXR-alpha and LIMS1

LXR-alpha and a fragment of LIMS1

A fragment of LXR-alpha and a fragment of LIMS1

TABLE 4

Protein Complexes LXR-alpha/PN7771 Interaction

Oxysterol liver X receptor alpha (LXR-alpha) and PN7771

A fragment of LXR-alpha and PN7771

LXR-alpha and a fragment of PN7771

A fragment of LXR-alpha and a fragment of PN7771

TABLE 5

Protein Complexes LXR-alpha/Homer-3 Interaction

Oxysterol liver X receptor alpha (LXR-alpha) and Homer-3

A fragment of LXR-alpha and Homer-3

LXR-alpha and a fragment of Homer-3

A fragment of LXR-alpha and a fragment of Homer-3

TABLE 6

Protein Complexes LXR-alpha/RACK1 Interaction

Oxysterol liver X receptor alpha (LXR-alpha) and RACK1

A fragment of LXR-alpha and RACK1

LXR-alpha and a fragment of RACK1

A fragment of LXR-alpha and a fragment of RACK1

TABLE 7

Protein Complexes LXR-alpha/EIF3S1 Interaction

Oxysterol liver X receptor alpha (LXR-alpha) and EIF3S1

A fragment of LXR-alpha and EIF3S1

LXR-alpha and a fragment of EIF3S1

A fragment of LXR-alpha and a fragment of EIF3S1

TABLE 8

Protein Complexes LXR-alpha/PSMD11 Interaction

Oxysterol liver X receptor alpha (LXR-alpha) and PSMD11
A fragment of LXR-alpha and PSMD11
LXR-alpha and a fragment of PSMD11
A fragment of LXR-alpha and a fragment of PSMD11

TABLE 9

Protein Complexes LXR-alpha/KIAA0610 Interaction

Oxysterol liver X receptor alpha (LXR-alpha) and KIAA0610
A fragment of LXR-alpha and KIAA0610
LXR-alpha and a fragment of KIAA0610
A fragment of LXR-alpha and a fragment of KIAA0610

TABLE 10

Protein Complexes LXR-alpha/CIR Interaction

Oxysterol liver X receptor alpha (LXR-alpha) and CIR
A fragment of LXR-alpha and CIR
LXR-alpha and a fragment of CIR
A fragment of LXR-alpha and a fragment of CIR

[0017] The involvement of above interactions in particular pathways is as follows.

[0018] Many cellular proteins exert their function by interacting with other proteins in the cell. Examples of this are found in the formation of multiprotein complexes and the association of enzymes with their substrates. It is widely believed that a great deal of information can be gained by understanding individual protein-protein interactions, and that this is useful in identifying complex networks of interacting proteins that participate in the workings of normal cellular functions. Ultimately, the knowledge gained by characterizing these networks can lead to valuable insight into the causes of human diseases and can eventually lead to the development of therapeutic strategies. The yeast two-hybrid assay is a powerful tool for determining protein-protein interactions and it has been successfully used for studying human disease pathways. In one variation of this technique, a protein of interest (or a portion of that protein) is expressed in a

population of yeast cells that collectively contain all protein sequences. Yeast cells that possess protein sequences that interact with the protein of interest are then genetically selected, and the identity of those interacting proteins are determined by DNA sequencing. Thus, proteins that can be demonstrated to interact with a protein known to be involved in a human disease are therefore also implicated in that disease. Proteins identified in the first round of two-hybrid screening can be subsequently used in a second round of two-hybrid screening, allowing the identification of multiple proteins in the complex network of interactions in a disease pathway.

[0019] Nuclear hormone receptors play important roles in development, reproduction, and physiology by altering gene transcription in response to hormonal signals (Whitfield et al., 1999; Klein-Hitpass et al., 1998). Misregulation of hormone receptor signaling pathways is responsible for a variety of diseases. For example, aldosterone and its receptor (the mineralocorticoid receptor, MCR) are involved in hypertension and congestive heart failure (Duprez et al., 2000), and it has recently been shown that a missense mutation in MCR that alters its ligand specificity is responsible for pregnancy-exacerbated hypertension (Geller et al., 2000). Likewise, glucocorticoids and the glucocorticoid receptor (GR) have been implicated in chronic inflammation and arthritis (Banres, 1998), and the oxysterol liver receptor (LXR), farnesoid X receptor (FXR), and other nuclear receptors are involved in cholesterol homeostasis and atherogenesis (Schroepfer, 2000; Haynes et al., 2000; Brown and Jessup, 1999)

[0020] Collectively, the nuclear receptor superfamily is responsive to a wide variety of ligands. Nuclear hormone receptors share several important structural features, including a variable N-terminal region, a conserved central DNA-binding domain, a variable hinge region, and a conserved C-terminal ligand-binding domain (Moras and Gronemeyer, 1998; Mangelsdorf et al., 1995). Despite this conserved structural organization, interactions between ligands and receptors are remarkably specific. Hormone binding results in conformational changes in the receptor, allowing binding to specific DNA sequences (hormone response elements, HREs) in target gene promoters resulting in changes in target gene transcription. Interaction of nuclear hormone receptors with accessory proteins determines whether the receptor activates or represses transcription. Receptors can recruit coactivators that remodel chromatin and stabilize the RNA polymerase machinery, or alternatively can interact with factors that condense chromatin structure and inactivate gene expression (Wolffe et al., 1997). Furthermore, binding of a nuclear hormone receptor to other cellular proteins can alter the subcellular localization of the receptor and control its ability to bind hormone and HREs (DeFranco et al., 1998). Clearly, identification of factors with which nuclear

hormone receptors interact is vital to understanding the process by which hormonal signals are transduced into transcriptional responses. In addition, identification of receptor-interacting proteins will increase the repertoire of potential targets for therapeutic intervention in the treatment of diseases due to defects involving nuclear hormone signaling.

[0021] The oxysterol liver X receptor alpha (LXRa) was used in yeast two-hybrid searches to identify novel protein-protein interactions. Here, we describe ten new interactors for LXRa. The first four interactors are involved in cell adhesion and cellular architecture. The first of these is the actin-binding protein utrophin. Utrophin is an autosomal gene that is similar to dystrophin, the gene famous for its role in Duchenne's muscular dystrophy. Unlike, dystrophin, however, utrophin appears to be expressed in a wide variety of adult tissues. Dystrophin and the dystrophin-related proteins contain spectrin repeats and likely play a role in anchoring the cytoskeleton to the plasma membrane by their actin-binding activities. The second interactor is the adhesion plaque protein zyxin, also involved in anchoring the cytoskeleton. Zyxin is a phosphoprotein that contains three LIM domains and two proline-rich regions. The interaction of LXRa with zyxin is reminiscent of the interaction we have identified between the farnesoid X-activated receptor and the LIM domain cytoskeletal protein Paxillin. Third, LXRa interacts with the novel protein PN7771, which is highly related (greater than 90% amino acid identity) to Ninein. Ninein is a centrosome-associated protein that interacts with human glycogen synthase kinase 3beta (GSK-3beta) (Hong et al., 2000), is localized to the pericentriolar matrix of the centrosome, and reacts with centrosomal autoantibody sera (Mack et al., 1998). PN7771 contains predicted calcium-binding EF hand motifs, a potential nuclear localization signal, a basic region-leucine zipper motif, a spectrin repeat, coiled-coil motifs, and Glu- and Gln-rich regions. Taken together, these interactions suggest that LXRa may be involved in cellular signaling events in response to cellular adhesion or other extracellular stimuli, and that the trans-activating ability of LXRa may be regulated by its interaction with these proteins.

[0022] Several LXRa interactors are involved in signal transduction pathways. The first is the neuronal immediate early protein homer-3. Homer proteins bind to the C-terminal tails of metabotropic glutamate receptors and play a role in their targeting and regulation; the metabotropic glutamate receptors, in turn, participate in the influx of intracellular calcium. Since LXR-alpha binds to homer-3, it is possible that LXRa may also be involved in calcium release. Alternatively, LXR-alpha could be modulated in some way by homer-3 in a manner analogous to the way in which the metabotropic glutamate receptors are regulated. The second protein, RACK1 (receptor of activated protein kinase C 1), is a WD repeat-containing protein that functions as an intracellular

receptor to localize PKC to the cytoskeleton. The interaction between RACK1 and LXR-alpha suggests that LXR-alpha may be capable of localizing to the cytoskeleton via its association with RACK1. The next interactor is the LIM-domain protein LIMS1. LIMS1 has been implicated in integrin-linked kinase signaling, and it has been shown to interact with the SH3 and SH2 domain-containing adaptor protein NCK2 (Tu et al. 1998). Taken together, these findings suggest the involvement of LXRa in a variety of signal transduction pathways; whether LXRa activity is regulated by interaction with these proteins, or vice versa, remains to be determined.

[0023] Two LXRa interactors involved in protein metabolism were identified: the proteasome subunit PSMD11, which is involved in protein turnover, and the translation initiation factor EIF3S1, which is involved in protein synthesis. The interaction of these proteins with LXRa suggests that nuclear hormone receptors may be involved directly with protein production and stability, in addition to transcriptional regulation.

[0024] An interaction between LXRa and the potential transmembrane protein KIAA0610 was identified. KIAA0610 is hypothetical protein fragment 686 amino acids in length. Predicted structural motifs include four possible transmembrane domains and a coiled-coil domain. KIAA0610 displays weak homology (~24% amino acid identity over 360-430 residues) to *Drosophila* and *C. elegans* proteins of unknown function. EST analysis suggests expression in a variety of tissues.

[0025] Finally, and interaction between LXRa and the transcription factor CIR was identified. CIR has been demonstrated to interact with the CBF1 transcription factor as well as histone deacetylase HD2 and Sin3-associated protein 30kD (Hsieh et al., 1999). It has been proposed that CIR acts as a linker between CBF1 and the histone deacetylase complex. Similarly, the interaction of LXRa with CIR suggests CIR may link LXRa with the histone deacetylase machinery. In support of a functional role between nuclear receptors and CIR, we have also identified an interaction between CIR and the estrogen receptor ER-beta.

[0026] The proteins disclosed in the present invention were found to interact with their corresponding proteins in the yeast two-hybrid system. Because of the involvement of the corresponding proteins in the physiological pathways disclosed herein, the proteins disclosed herein also participate in the same physiological pathways. Therefore, the present invention provides a list of uses of these proteins and DNA encoding these proteins for the development of diagnostic and therapeutic tools useful in the physiological pathways. This list includes, but is not limited to, the following examples.

Two-Hybrid System

[0027] The principles and methods of the yeast two-hybrid system have been described in detail elsewhere (e.g., Bartel and Fields, 1997; Bartel et al., 1993; Fields and Song, 1989; Chevray and Nathans, 1992). The following is a description of the use of this system to identify proteins that interact with a protein of interest.

[0028] The target protein is expressed in yeast as a fusion to the DNA-binding domain of the yeast Gal4p. DNA encoding the target protein or a fragment of this protein is amplified from cDNA by PCR or prepared from an available clone. The resulting DNA fragment is cloned by ligation or recombination into a DNA-binding domain vector (e.g., pGBT9, pGBT.C, pAS2-1) such that an in-frame fusion between the Gal4p and target protein sequences is created.

[0029] The target gene construct is introduced, by transformation, into a haploid yeast strain. A library of activation domain fusions (i.e., adult brain cDNA cloned into an activation domain vector) is introduced by transformation into a haploid yeast strain of the opposite mating type. The yeast strain that carries the activation domain constructs contains one or more Gal4p-responsive reporter gene(s), whose expression can be monitored. Examples of some yeast reporter strains include Y190, PJ69, and CBY14a. An aliquot of yeast carrying the target gene construct is combined with an aliquot of yeast carrying the activation domain library. The two yeast strains mate to form diploid yeast and are plated on media that selects for expression of one or more Gal4p-responsive reporter genes. Colonies that arise after incubation are selected for further characterization.

[0030] The activation domain plasmid is isolated from each colony obtained in the two-hybrid search. The sequence of the insert in this construct is obtained by the dideoxy nucleotide chain termination method. Sequence information is used to identify the gene/protein encoded by the activation domain insert via analysis of the public nucleotide and protein databases. Interaction of the activation domain fusion with the target protein is confirmed by testing for the specificity of the interaction. The activation domain construct is co-transformed into a yeast reporter strain with either the original target protein construct or a variety of other DNA-binding domain constructs. Expression of the reporter genes in the presence of the target protein but not with other test proteins indicates that the interaction is genuine.

[0031] In addition to the yeast two-hybrid system, other genetic methodologies are available for the discovery or detection of protein-protein interactions. For example, a mammalian two-hybrid

system is available commercially (Clontech, Inc.) that operates on the same principle as the yeast two-hybrid system. Instead of transforming a yeast reporter strain, plasmids encoding DNA-binding and activation domain fusions are transfected along with an appropriate reporter gene (e.g., lacZ) into a mammalian tissue culture cell line. Because transcription factors such as the *Saccharomyces cerevisiae* Gal4p are functional in a variety of different eukaryotic cell types, it would be expected that a two-hybrid assay could be performed in virtually any cell line of eukaryotic origin (e.g., insect cells (SF9), fungal cells, worm cells, etc.). Other genetic systems for the detection of protein-protein interactions include the so-called SOS recruitment system (Aronheim et al., 1997).

Protein-protein interactions

[0032] Protein interactions are detected in various systems including the yeast two-hybrid system, affinity chromatography, co-immunoprecipitation, subcellular fractionation and isolation of large molecular complexes. Each of these methods is well characterized and can be readily performed by one skilled in the art. See, e.g., U.S. Patents No. 5,622,852 and 5,773,218, and PCT published applications No. WO 97/27296 and WO 99/65939, each of which are incorporated herein by reference.

[0033] The protein of interest can be produced in eukaryotic or prokaryotic systems. A cDNA encoding the desired protein is introduced in an appropriate expression vector and transfected in a host cell (which could be bacteria, yeast cells, insect cells, or mammalian cells). Purification of the expressed protein is achieved by conventional biochemical and immunochemical methods well known to those skilled in the art. The purified protein is then used for affinity chromatography studies: it is immobilized on a matrix and loaded on a column. Extracts from cultured cells or homogenized tissue samples are then loaded on the column in appropriate buffer, and non-binding proteins are eluted. After extensive washing, binding proteins or protein complexes are eluted using various methods such as a gradient of pH or a gradient of salt concentration. Eluted proteins can then be separated by two-dimensional gel electrophoresis, eluted from the gel, and identified by micro-sequencing. The purified proteins can also be used for affinity chromatography to purify interacting proteins disclosed herein. All of these methods are well known to those skilled in the art.

[0034] Similarly, both proteins of the complex of interest (or interacting domains thereof) can be produced in eukaryotic or prokaryotic systems. The proteins (or interacting domains) can be under control of separate promoters or can be produced as a fusion protein. The fusion protein

may include a peptide linker between the proteins (or interacting domains) which, in one embodiment, serves to promote the interaction of the proteins (or interacting domains). All of these methods are also well known to those skilled in the art.

[0035] Purified proteins of interest, individually or a complex, can also be used to generate antibodies in rabbit, mouse, rat, chicken, goat, sheep, pig, guinea pig, bovine, and horse. The methods used for antibody generation and characterization are well known to those skilled in the art. Monoclonal antibodies are also generated by conventional techniques. Single chain antibodies are further produced by conventional techniques.

[0036] DNA molecules encoding proteins of interest can be inserted in the appropriate expression vector and used for transfection of eukaryotic cells such as bacteria, yeast, insect cells, or mammalian cells, following methods well known to those skilled in the art. Transfected cells expressing both proteins of interest are then lysed in appropriate conditions, one of the two proteins is immunoprecipitated using a specific antibody, and analyzed by polyacrylamide gel electrophoresis. The presence of the binding protein (co-immunoprecipitated) is detected by immunoblotting using an antibody directed against the other protein. Co-immunoprecipitation is a method well known to those skilled in the art.

[0037] Transfected eukaryotic cells or biological tissue samples can be homogenized and fractionated in appropriate conditions that will separate the different cellular components. Typically, cell lysates are run on sucrose gradients, or other materials that will separate cellular components based on size and density. Subcellular fractions are analyzed for the presence of proteins of interest with appropriate antibodies, using immunoblotting or immunoprecipitation methods. These methods are all well known to those skilled in the art.

Disruption of protein-protein interactions

[0038] It is conceivable that agents that disrupt protein-protein interactions can be beneficial in many physiological disorders, including, but not-limited to NIDDM, AD and others disclosed herein. Each of the methods described above for the detection of a positive protein-protein interaction can also be used to identify drugs that will disrupt said interaction. As an example, cells transfected with DNAs coding for proteins of interest can be treated with various drugs, and co-immunoprecipitations can be performed. Alternatively, a derivative of the yeast two-hybrid system, called the reverse yeast two-hybrid system (Leanna and Hannink, 1996), can be used, provided that the two proteins interact in the straight yeast two-hybrid system.

Modulation of protein-protein interactions

[0039] Since the interactions described herein are involved in a physiological pathway, the identification of agents which are capable of modulating the interactions will provide agents which can be used to track physiological disorder or to use lead compounds for development of therapeutic agents. An agent may modulate expression of the genes of interacting proteins, thus affecting interaction of the proteins. Alternatively, the agent may modulate the interaction of the proteins. The agent may modulate the interaction of wild-type with wild-type proteins, wild-type with mutant proteins, or mutant with mutant proteins. Agents which may be used to modulate the protein interaction inlcude a peptide, an antibody, a nucleic acid, an antisense compound or a ribozyme. The nucleic acid may encode the antibody or the antisense compound. The peptide may be at least 4 amino acids of the sequence of either of the interacting proteins. Alternatively, the peptide may be from 4 to 30 amino acids (or from 8 to 20 amino acids) that is at least 75% identical to a contiguous span of amino acids of either of the interacting proteins. The peptide may be covalently linked to a transporter capable of increasing cellular uptake of the peptide. Examples of a suitable transporter include penetratins, *l*-Tat_{49-57}, *d*-Tat_{49-57}, retro-inverso isomers of *l*- or *d*-Tat_{49-57}, L-arginine oligomers, D- arginine oligomers, L-lysine oligomers, D-lysine oligomers, L-histine oligomers, D-histine oligomers, L-ornithine oligomers, D-ornithine oligomers, short peptide sequences derived from fibroblast growth factor, Galparan, and HSV-1 structural protein VP22, and peptoid analogs thereof. Agents can be tested using transfected host cells, cell lines, cell models or animals, such as described herein, by techniques well known to those of ordinary skill in the art, such as disclosed in U.S. Patents Nos. 5,622,852 and 5,773,218, and PCT published application Nos. WO 97/27296 and WO 99/65939, each of which are incorporated herein by reference. The modulating effect of the agent can be tested *in vivo* or *in vitro*. Agents can be provided for testing in a phage display library or a combinatorial library. Exemplary of a method to screen agents is to measure the effect that the agent has on the formation of the protein complex.

Mutation screening

[0040] The proteins disclosed in the present invention interact with one or more proteins known to be involved in a physiological pathway, such as in NIDDM, AD or pathways described herein. Mutations in interacting proteins could also be involved in the development of the physiological disorder, such as NIDDM, AD or disorders described herein, for example, through

a modification of protein-protein interaction, or a modification of enzymatic activity, modification of receptor activity, or through an unknown mechanism. Therefore, mutations can be found by sequencing the genes for the proteins of interest in patients having the physiological disorder, such as insulin, and non-affected controls. A mutation in these genes, especially in that portion of the gene involved in protein interactions in the physiological pathway, can be used as a diagnostic tool and the mechanistic understanding the mutation provides can help develop a therapeutic tool.

Screening for at-risk individuals

[0041] Individuals can be screened to identify those at risk by screening for mutations in the protein disclosed herein and identified as described above. Alternatively, individuals can be screened by analyzing the ability of the proteins of said individual disclosed herein to form natural complexes. Further, individuals can be screened by analyzing the levels of the complexes or individual proteins of the complexes or the mRNA encoding the protein members of the complexes. Techniques to detect the formation of complexes, including those described above, are known to those skilled in the art. Techniques and methods to detect mutations are well known to those skilled in the art. Techniques to detect the level of the complexes, proteins or mRNA are well known to those skilled in the art.

Cellular models of Physiological Disorders

[0042] A number of cellular models of many physiological disorders or diseases have been generated. The presence and the use of these models are familiar to those skilled in the art. As an example, primary cell cultures or established cell lines can be transfected with expression vectors encoding the proteins of interest, either wild-type proteins or mutant proteins. The effect of the proteins disclosed herein on parameters relevant to their particular physiological disorder or disease can be readily measured. Furthermore, these cellular systems can be used to screen drugs that will influence those parameters, and thus be potential therapeutic tools for the particular physiological disorder or disease. Alternatively, instead of transfecting the DNA encoding the protein of interest, the purified protein of interest can be added to the culture medium of the cells under examination, and the relevant parameters measured.

Animal models

[0043] The DNA encoding the protein of interest can be used to create animals that overexpress said protein, with wild-type or mutant sequences (such animals are referred to as "transgenic"), or animals which do not express the native gene but express the gene of a second animal (referred to as "transplacement"), or animals that do not express said protein (referred to as "knock-out"). The knock-out animal may be an animal in which the gene is knocked out at a determined time. The generation of transgenic, transplacement and knock-out animals (normal and conditioned) uses methods well known to those skilled in the art.

[0044] In these animals, parameters relevant to the particular physiological disorder can be measured. These parametes may include receptor function, protein secretion *in vivo* or *in vitro*, survival rate of cultured cells, concentration of particular protein in tissue homogenates, signal transduction, behavioral analysis, protein synthesis, cell cycle regulation, transport of compounds across cell or nuclear membranes, enzyme activity, oxidative stress, production of pathological products, and the like. The measurements of biochemical and pathological parameters, and of behavioral parameters, where appropriate, are performed using methods well known to those skilled in the art. These transgenic, transplacement and knock-out animals can also be used to screen drugs that may influence the biochemical, pathological, and behavioral parameters relevant to the particular physiological disorder being studied. Cell lines can also be derived from these animals for use as cellular models of the physiological disorder, or in drug screening.

Rational drug design

[0045] The goal of rational drug design is to produce structural analogs of biologically active polypeptides of interest or of small molecules with which they interact (e.g., agonists, antagonists, inhibitors) in order to fashion drugs which are, for example, more active or stable forms of the polypeptide, or which, e.g., enhance or interfere with the function of a polypeptide *in vivo*. Several approaches for use in rational drug design include analysis of three-dimensional structure, alanine scans, molecular modeling and use of anti-id antibodies. These techniques are well known to those skilled in the art. Such techniques may include providing atomic coordinates defining a three-dimensional structure of a protein complex formed by said first polypeptide and said second polypeptide, and designing or selecting compounds capable of interfering with the interaction between a first polypeptide and a second polypeptide based on said atomic coordinates.

[0046] Following identification of a substance which modulates or affects polypeptide activity, the substance may be further investigated. Furthermore, it may be manufactured and/or used in preparation, i.e., manufacture or formulation, or a composition such as a medicament, pharmaceutical composition or drug. These may be administered to individuals.

[0047] A substance identified as a modulator of polypeptide function may be peptide or non-peptide in nature. Non-peptide "small molecules" are often preferred for many *in vivo* pharmaceutical uses. Accordingly, a mimetic or mimic of the substance (particularly if a peptide) may be designed for pharmaceutical use.

[0048] The designing of mimetics to a known pharmaceutically active compound is a known approach to the development of pharmaceuticals based on a "lead" compound. This approach might be desirable where the active compound is difficult or expensive to synthesize or where it is unsuitable for a particular method of administration, e.g., pure peptides are unsuitable active agents for oral compositions as they tend to be quickly degraded by proteases in the alimentary canal. Mimetic design, synthesis and testing is generally used to avoid randomly screening large numbers of molecules for a target property.

[0049] Once the pharmacophore has been found, its structure is modeled according to its physical properties, e.g., stereochemistry, bonding, size and/or charge, using data from a range of sources, e.g., spectroscopic techniques, x-ray diffraction data and NMR. Computational analysis, similarity mapping (which models the charge and/or volume of a pharmacophore, rather than the bonding between atoms) and other techniques can be used in this modeling process.

[0050] A template molecule is then selected, onto which chemical groups that mimic the pharmacophore can be grafted. The template molecule and the chemical groups grafted thereon can be conveniently selected so that the mimetic is easy to synthesize, is likely to be pharmacologically acceptable, and does not degrade *in vivo*, while retaining the biological activity of the lead compound. Alternatively, where the mimetic is peptide-based, further stability can be achieved by cyclizing the peptide, increasing its rigidity. The mimetic or mimetics found by this approach can then be screened to see whether they have the target property, or to what extent it is exhibited. Further optimization or modification can then be carried out to arrive at one or more final mimetics for *in vivo* or clinical testing.

Diagnostic Assays

[0051] The identification of the interactions disclosed herein enables the development of diagnostic assays and kits, which can be used to determine a predisposition to or the existence of a physiological disorder. In one aspect, one of the proteins of the interaction is used to detect the presence of a "normal" second protein (i.e., normal with respect to its ability to interact with the first protein) in a cell extract or a biological fluid, and further, if desired, to detect the quantitative level of the second protein in the extract or biological fluid. The absence of the "normal" second protein would be indicative of a predisposition or existence of the physiological disorder. In a second aspect, an antibody against the protein complex is used to detect the presence and/or quantitative level of the protein complex. The absence of the protein complex would be indicative of a predisposition or existence of the physiological disorder.

Nucleic Acids and Proteins

[0052] A nucleic acid or fragment thereof has substantial identity with another if, when optimally aligned (with appropriate nucleotide insertions or deletions) with the other nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 60% of the nucleotide bases, usually at least about 70%, more usually at least about 80%, preferably at least about 90%, more preferably at least about 95% of the nucleotide bases, and more preferably at least about 98% of the nucleotide bases. A protein or fragment thereof has substantial identity with another if, optimally aligned, there is an amino acid sequence identity of at least about 30% identity with an entire naturally-occurring protein or a portion thereof, usually at least about 70% identity, more ususally at least about 80% identity, preferably at least about 90% identity, more preferably at least about 95% identity, and most preferably at least about 98% identity.

[0053] Identity means the degree of sequence relatedness between two polypeptide or two polynucleotides sequences as determined by the identity of the match between two strings of such sequences. Identity can be readily calculated. While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (*Computational Molecular Biology*, Lesk, A. M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D. W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data*, Part I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M

Stockton Press, New York, 1991). Methods commonly employed to determine identity between two sequences include, but are not limited to those disclosed in *Guide to Huge Computers*, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipman, D., *SIAM J Applied Math.* **48**:1073 (1988). Preferred methods to determine identity are designed to give the largest match between the two sequences tested. Such methods are codified in computer programs. Preferred computer program methods to determine identity between two sequences include, but are not limited to, GCG (Genetics Computer Group, Madison Wis.) program package (Devereux, J., et al., *Nucleic Acids Research* **12**(1).387 (1984)), BLASTP, BLASTN, FASTA (Altschul et al. (1990); Altschul et al. (1997)). The well-known Smith Waterman algorithm may also be used to determine identity.

[0054] Alternatively, substantial homology or similarity exists when a nucleic acid or fragment thereof will hybridize to another nucleic acid (or a complementary strand thereof) under selective hybridization conditions, to a strand, or to its complement. Selectivity of hybridization exists when hybridization which is substantially more selective than total lack of specificity occurs. Nucleic acid hybridization will be affected by such conditions as salt concentration, temperature, or organic solvents, in addition to the base composition, length of the complementary strands, and the number of nucleotide base mismatches between the hybridizing nucleic acids, as will be readily appreciated by those skilled in the art. Stringent temperature conditions will generally include temperatures in excess of 30^{o}C, typically in excess of 37^{o}C, and preferably in excess of 45^{o}C. Stringent salt conditions will ordinarily be less than 1000 mM, typically less than 500 mM, and preferably less than 200 mM. However, the combination of parameters is much more important than the measure of any single parameter. See, e.g., Asubel, 1992; Wetmur and Davidson, 1968.

[0055] The terms "isolated", "substantially pure", and "substantially homogeneous" are used interchangeably to describe a protein or polypeptide which has been separated from components which accompany it in its natural state. A monomeric protein is substantially pure when at least about 60 to 75% of a sample exhibits a single polypeptide sequence. A substantially pure protein will typically comprise about 60 to 90% W/W of a protein sample, more usually about 95%, and preferably will be over about 99% pure. Protein purity or homogeneity may be indicated by a number of means well known in the art, such as polyacrylamide gel electrophoresis of a protein sample, followed by visualizing a single polypeptide band upon staining the gel. For certain

purposes, higher resolution may be provided by using HPLC or other means well known in the art which are utilized for purification.

[0056] Large amounts of the nucleic acids of the present invention may be produced by (a) replication in a suitable host or transgenic animals or (b) chemical synthesis using techniques well known in the art. Constructs prepared for introduction into a prokaryotic or eukaryotic host may comprise a replication system recognized by the host, including the intended polynucleotide fragment encoding the desired polypeptide, and will preferably also include transcription and translational initiation regulatory sequences operably linked to the polypeptide encoding segment. Expression vectors may include, for example, an origin of replication or autonomously replicating sequence (ARS) and expression control sequences, a promoter, an enhancer and necessary processing information sites, such as ribosome-binding sites, RNA splice sites, polyadenylation sites, transcriptional terminator sequences, and mRNA stabilizing sequences. Secretion signals may also be included where appropriate which allow the protein to cross and/or lodge in cell membranes, and thus attain its functional topology, or be secreted from the cell. Such vectors may be prepared by means of standard recombinant techniques well known in the art.

[0057] The nucleic acid or protein may also be incorporated on a microarray. The preparation and use of microarrays are well known in the art. Generally, the microarray may contain the entire nucleic acid or protein, or it may contain one or more fragments of the nucleic acid or protein. Suitable nucleic acid fragments may include at least 17 nucleotides, at least 21 nucleotides, at least 30 nucleotides or at least 50 nucleotides of the nucleic acid sequence, particularly the coding sequence. Suitable protein fragments may include at least 4 amino acids, at least 8 amino acids, at least 12 amino acids, at least 15 amino acids, at least 17 amino acids or at least 20 amino acids. Thus, the present invention is also directed to such nucleic acid and protein fragments.

EXAMPLES

[0058] The present invention is further detailed in the following Examples, which are offered by way of illustration and are not intended to limit the invention in any manner. Standard techniques well known in the art or the techniques specifically described below are utilized.

EXAMPLE 1

Yeast Two-Hybrid System

[0059] The principles and methods of the yeast two-hybrid systems have been described in detail (Bartel and Fields, 1997). The following is thus a description of the particular procedure that we used, which was applied to all proteins.

[0060] The cDNA encoding the bait protein was generated by PCR from brain cDNA. Gene-specific primers were synthesized with appropriate tails added at their 5' ends to allow recombination into the vector pGBTQ. The tail for the forward primer was 5'-GCAGGAAACAGCTATGACCATACAGTCAGCGGCCGCCACC-3' (SEQ ID NO:1) and the tail for the reverse primer was 5'-ACGGCCAGTCGCGTGGAGTGTTATGTCATGCGGCCGCTA-3' (SEQ ID NO:2). The tailed PCR product was then introduced by recombination into the yeast expression vector pGBTQ, which is a close derivative of pGBTC (Bartel et al., 1996) in which the polylinker site has been modified to include M13 sequencing sites. The new construct was selected directly in the yeast J693 for its ability to drive tryptophane synthesis (genotype of this strain: Mat α, ade2, his3, leu2, trp1, URA3::GAL1-lacZ LYS2::GAL1-HIS3 gal4del gal80del cyhR2). In these yeast cells, the bait is produced as a C-terminal fusion protein with the DNA binding domain of the transcription factor Gal4 (amino acids 1 to 147). A total human brain (37 year-old male Caucasian) cDNA library cloned into the yeast expression vector pACT2 was purchased from Clontech (human brain MATCHMAKER cDNA, cat. # HL4004AH), transformed into the yeast strain J692 (genotype of this strain: Mat a, ade2, his3, leu2, trp1, URA3::GAL1-lacZ LYS2::GAL1-HIS3 gal4del gal80del cyhR2), and selected for the ability to drive leucine synthesis. In these yeast cells, each cDNA is expressed as a fusion protein with the transcription activation domain of the transcription factor Gal4 (amino acids 768 to 881) and a 9 amino acid hemagglutinin epitope tag. J693 cells (Mat α type) expressing the bait were then mated with J692 cells (Mat a type) expressing proteins from the brain library. The resulting diploid yeast cells expressing proteins interacting with the bait protein were selected for the ability to synthesize tryptophan, leucine, histidine, and β-galactosidase. DNA was prepared from each clone, transformed by electroporation into *E. coli* strain KC8 (Clontech KC8 electrocompetent cells, cat. # C2023-1), and the cells were selected on ampicillin-containing plates in the absence of either tryptophane (selection for the bait plasmid) or leucine (selection for the brain library plasmid). DNA for both plasmids was prepared and sequenced by di-deoxynucleotide chain termination method. The identity of the bait cDNA insert was confirmed and the cDNA insert from the brain library plasmid was identified using BLAST program against public

nucleotides and protein databases. Plasmids from the brain library (preys) were then individually transformed into yeast cells together with a plasmid driving the synthesis of lamin fused to the Gal4 DNA binding domain. Clones that gave a positive signal after β-galactosidase assay were considered false-positives and discarded. Plasmids for the remaining clones were transformed into yeast cells together with plasmid for the original bait. Clones that gave a positive signal after β-galactosidase assay were considered true positives.

EXAMPLE 2

Identification of LXR-alpha/Utrophin Interaction

[0061] A yeast two-hybrid system as described in Example 1 using amino acids 95-277 of LXR-alpha (GenBank (GB) accession no. U22662) as bait was performed. One clone that was identified by this procedure included amino acids 2443-2650 of utophin (GB accession no. X15488).

EXAMPLE 3

Identification of LXR-alpha/PN7771 Interaction

[0062] A yeast two-hybrid system as described in Example 1 using amino acids 95-277 of LXR-alpha (GB accession no. U22662) as bait was performed. One clone that was identified by this procedure included amino acids 1747-2047 of PN7771. The DNA sequence and the predicted protein sequence for PN7771 are set forth in Tables 11 and 12, respectively.

TABLE 11

Nucleotide Sequence of PN7771

cttattttgaaaacatttacatagtgattagttaacccaacagaccaatcctgggaagacagccagagcctgcagcaccttagtaacagaaaaactg
ataattaggagaagagacctgtccaagaccaggaacctggaccaaaattgtgccatgttgctttactttaatgagtggccccagtaaaaactgagct
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gagaaataccatacagataccttaaatgtatgcatttgtgcaacaatttttgagaaggtgagtggcaatttataatttagttggcaatttataatagaact
tatagcttttaaaagacttttaaagacattaaatgtaaacttaaaaatgtttagatcttgtttcaaactttacaatagcattcttcaaaatattaagttatatat
tttataggcatttagttgcttattaaaagcactgattttcaaacttttgatttaagaacaattatttaagatcgtctcagaagatgggatcttcgtttcaaga
aaagggaatcaagtttgcctttgagataatacgttacactaagaaaaggaaaatgtggatagtaaaacccacctctctcatcctattgtactctcttct
gcttttagaagcctgcacttaagcttagatttgtgaagggagagtagaaggggagaagtagaaccacagtgttttatttatttttctaaaactcttact
aaatccagatttttaaactgttttaaatgtgaattcttcccagaaatttcaatgcattgcatatttagccttcggcatatttttcatgaatagatcatgaagt
cataggcttccaaggcataggaagagatcttgcaggtctagtattttaataatgcactattacccagggcagatattatgagaaactgtttcttctctaa
gggtttatggcagactttgctttttaacatgtgagaaatgaatttttattttgtgatttatgtgatttctttgctgagtgaaggaaaggagaaattgttgc
tattgtcagcatcttaaaggtatttccagtcaaggcaaggctaagtgctttgtgatagtattaagcaagtcatgttttgaatggattacctgtagtgactc
attggaatgatataattatacaagtaatgccaaaaaccaagtcaaagcctaattaaccaaagcactcatttaaaaatcatcatgtttggacctatctgg
acctctcagcactgtaaaatagttttggttttgtggcatatgaatagctgtttaacaaatcaaagttagctttttgcttctcagctttttgggcaatacaag
ttaagttcttaatggggagacattatcatggcatgacttaagggaacattggtttgtgaaggaaaaacagattatctaaagccatctctatgtttctgttc

agataaagattaatgagttctgtgtttatatcagctttgtatatttcatcttagccattctatcctagaaagattttaatgtgagcttaagatgtaaataaata
attttgcaaacatgaaaaaaaaaaaaaaaa (SEQ ID NO:3)

TABLE 12

Predicted Amino Acid Sequence of PN7771

MAEVTVPRVYVVFGIHCIMAKASSDVQVSGFHRKIQHVKNELCHMLSLEEVAPVLQQTLL
QDNLLGRVHFDQFKEALILILSRTLSNEEHFQEPDCSLEAQPKYVRGGKRYGRRSLPEFQES
VEEFPEVTVIEPLDEEARPSHIPAGDCSEHWKTQRSEEYEAEGQLRFWNPDDLNASQSGSSP
PQDWIEEKLQEVCEDLGITRDGHLNRKKLVSICEQYGLQNVDGEMLEEVFHNLDPDGTMS
VEDFFYGLFKNGKSLTPSASTPYRQLKRHLSMQSFDESGRRTTTSSAMTSTIGFRVFSCLDD
GMGHASVERILDTWQEEGIENSQEILKALDFSLDGNINLTELTLALENELLVTKNSIHQAAL
ASFKAEIRHLLERVDQVVREKEKLRSDLDKAEKLKSLMASEVDDHHAAIERRNEYNLRKL
DGEYKERIAALKNELRKEREQILQQAGKQRLELEQEIEKAKTEENYIRDRLALSLKENSRLE
NELLENAEKLAEYENLTNKLQRNLENVLAEKFGDLDPSSAEFFLQEERLTQMRNEYERQCR
VLQDQVDELQSELEEYRAQGRVLRLPLKNSPSEEVEANSGGIEPEHGLGSEECNPLNMSIEA
ELVIEQMKEQHHRDICCLRLELEDKVRHYEKQLDETVVSCKKAQENMKQRHENETRTLEK
QISDLKNEIAELQGQAAVLKEAHHEATCRHEEEKKQLQVKLEEEKTHLQEKLRLQHEMEL
KARLTQAQASFEREREGLQSSAWTEEKVRGLTQELEQFHQEQLTSLVEKHTLEKEELRKEL
LEKHQRELQEGREKMETECNRRTSQIEAQFQSDCQKVTERCESALQSLEGRYRQELKDLQE
QQREEKSQWEFEKDELTQECAEAQELLKETLKREKTTSLVLTQEREMLEKTYKEHLNSMV
VERQQLLQDLEDLRNVSETQQSLLSDQILELKSSHKRELREREEVLCQAGASEQLASQRLER
LEMEHDQERQEMMSKLLAMENIHKATCETADRERAEMSTEISRLQSKIKEMQQATSPLSM
LQSGCQVIGEEEVEGDGALSLLQQGEQLLEENGDVLLSLQRAHEQAVKENVKMATEISRLQ
QRLQKLEPGLVMSSCLDEPATEFFGNTAEQTEQFLQQNRTKQVEGVTRRHVLSDLEDDEVR
DLGSTGTSSVQRQEVKIEESEASVEGFSELENSEETRTESWELKNQISQLQEQLMMLCADCD
RASEKKQDLLFDVSVLKKKLKMLERIPEASPKYKLLYEDVSRENDCLQEELRMMETRYDE
ALENNKELTAEVFRLQDELKKMEEVTETFLSLEKSYDEVKIENEGLNVLVLRLQGKIEKLQE
SVVQRCDCCLWEASLENLEIEPDGNILQLNQTLEECVPRVRSVHHVIEECKQENQYLEGNT
QLLEKVKAHEIAWLHGTIQTHQERPRVQNQVILEENTTLLGFQDKHFQHQATIAELELEKTK
LQELTRKLKERVTILVKQKDVLSHGEKEEELKAMMHDLQITCSEMQQKVELLRYESEKLQ
QENSILRNEITTLNEEDSISNLKLGTLNGSQEEMWQKTETVKQENAAVQKMVENLKKQISE
LKIKNQQLDLENTELSQKNSQNQEKLQELNQRLTEMLCQKEKEPGNSALEEREQEKFNLKE
ELERCKVQSSTLVSSLEAELSEVKIQTHIVQQENHLLKDELEKMKQLHRCPDLSDFQQKISS
VLSYNEKLLKEKEALSEELNSCVDKLAKSSLLEHRIATMKQEQKSWEHQSASLKSQLVASQ
EKVQNLEDTVQNVNLQMSRMKSDLRVTQQEKEALKQEVMSLHKQLQNAGGKSWAPEIAT
HPSGLHNQQKRLSWDKLDHLMNEEQQLLWQENERLQTMVQNTKAELTHSREKVRQLESN
LLPKHQKHLNPSGTMNPTEQEKLSLKRECDQFQKEQSPANRKVSQMNSLEQELETIHLENE
GLKKKQVKLDEQLMEMQHLRSTATPSPSPHAWDLQLLQQQACPMVPREQFLQLQRQLLQ
AERINQHLQEELENRTSETNTPQGNQEQLVTVMEERMIEVEQKLKLVKRLLQEKVNQLKEQ
LCKNTKADAMVKDLYVENAQLLKALEVTEQRQKTAEKKNYLLEEKIASLSNIVRNLTPAPL
TSTPPLRS (SEQ ID NO:4)

EXAMPLES 4-12

Identification of Protein-Protein Interactions

[0063] A yeast two-hybrid system as described in Example 1 using amino acids of the bait as set forth in Table 13 was performed. The clone that was identified by this procedure for each bait is set forth in Table 13 as the prey. The "AA" refers to the amino acids of the bait or prey. The "NUC" refers to the nucleotides of the bait or prey. The Accession numbers refer to GB: GenBank accession numbers.

TABLE 13

Ex.	BAIT	ACCESSION	COORDINATES	PREY	ACCESSION	COORDINATES
4	LXR-alpha	GB: U22662	AA: 95-156	zyxin	GB: X94991	AA 323-572
5	LXR-alpha	GB: U22662	AA 95-156	LIMS1	GB: U09284	AA 25-90
6	LXR-alpha	GB: U22662	AA 95-277	Homer-3	GB: NM_004838	AA 182-354
7	LXR-alpha	GB: U22662	AA 95-277	RACK1	GB: M24194	AA 178-317
8	LXR-alpha	GB: U22662	AA 257-448	EIF3S1	GB: NM_003758	AA 94-254
9	LXR-alpha	GB: U22662	AA 156-448	EIF3S1	GB: NM_003758	AA 94-254
10	LXR-alpha	GB: U22662	AA 95-277	PSMD11	GB: NM_002815	AA 87-422
11	LXR-alpha	GB: U22662	AA 257-448	KIAA0610	GB: AB011182	AA 36-245
12	LXR-alpha	GB: U22662	AA 156-448	CIR	GB: U03644	AA 226-450

EXAMPLE 13

Generation of Polyclonal Antibody Against Protein Complexes

[0064] As shown above, LXR-alpha interacts with utrophin to form a complex. A complex of the two proteins is prepared, e.g., by mixing purified preparations of each of the two proteins. If desired, the protein complex can be stabilized by cross-linking the proteins in the complex, by methods known to those of skill in the art. The protein complex is used to immunize rabbits and mice using a procedure similar to that described by Harlow et al. (1988). This procedure has been shown to generate Abs against various other proteins (for example, see Kraemer et al., 1993).

[0065] Briefly, purified protein complex is used as immunogen in rabbits. Rabbits are immunized with 100 μg of the protein in complete Freund's adjuvant and boosted twice in three-week intervals, first with 100 μg of immunogen in incomplete Freund's adjuvant, and followed by 100 μg of immunogen in PBS. Antibody-containing serum is collected two weeks thereafter. The antisera is preadsorbed with LXR-alpha and utrophin, such that the remaining antisera comprises antibodies which bind conformational epitopes, i.e., complex-specific epitopes, present on the LXR-alpha-utrophin complex but not on the monomers.

[0066] Polyclonal antibodies against each of the complexes set forth in Tables 1-10 are prepared in a similar manner by mixing the specified proteins together, immunizing an animal and isolating antibodies specific for the protein complex, but not for the individual proteins.

[0067] Polyclonal antibodies against the protein set forth in Table 12 are prepared in a similar manner by immunizing an animal with the protein and isolating antibodies specific for the protein.

EXAMPLE 14

Generation of Monoclonal Antibodies Specific for Protein Complexes

[0068] Monoclonal antibodies are generated according to the following protocol. Mice are immunized with immunogen comprising LXR-alpha/utrophin complexes conjugated to keyhole limpet hemocyanin using glutaraldehyde or EDC as is well known in the art. The complexes can be prepared as described in Example 13, and may also be stabilized by cross-linking. The immunogen is mixed with an adjuvant. Each mouse receives four injections of 10 to 100 μg of immunogen, and after the fourth injection blood samples are taken from the mice to determine if the serum contains antibody to the immunogen. Serum titer is determined by ELISA or RIA. Mice

with sera indicating the presence of antibody to the immunogen are selected for hybridoma production.

[0069] Spleens are removed from immune mice and a single-cell suspension is prepared (Harlow et al., 1988). Cell fusions are performed essentially as described by Kohler et al. (1975). Briefly, P3.65.3 myeloma cells (American Type Culture Collection, Rockville, MD) or NS-1 myeloma cells are fused with immune spleen cells using polyethylene glycol as described by Harlow et al. (1988). Cells are plated at a density of $2x10^5$ cells/well in 96-well tissue culture plates. Individual wells are examined for growth, and the supernatants of wells with growth are tested for the presence of LXR-alpha/utrophin complex-specific antibodies by ELISA or RIA using LXR-alpha/utrophin complex as target protein. Cells in positive wells are expanded and subcloned to establish and confirm monoclonality.

[0070] Clones with the desired specificities are expanded and grown as ascites in mice or in a hollow fiber system to produce sufficient quantities of antibodies for characterization and assay development. Antibodies are tested for binding to LXR-alpha alone or to utrophin alone, to determine which are specific for the LXR-alpha/utrophin complex as opposed to those that bind to the individual proteins.

[0071] Monoclonal antibodies against each of the complexes set forth in Tables 1-10 are prepared in a similar manner by mixing the specified proteins together, immunizing an animal, fusing spleen cells with myeloma cells and isolating clones which produce antibodies specific for the protein complex, but not for the individual proteins.

[0072] Monoclonal antibodies against the protein set forth in Table 12 are prepared in a similar manner by immunizing an animal with the protein, fusing spleen cells with myeloma cells and isolating clones which produce antibodies specific for the protein.

EXAMPLE 15

In vitro Identification of Modulators for Protein-Protein Interactions

[0073] The present invention is useful in screening for agents that modulate the interaction of LXR-alpha and utrophin. The knowledge that LXR-alpha and utrophin form a complex is useful in designing such assays. Candidate agents are screened by mixing LXR-alpha and utrophin (a) in the presence of a candidate agent, and (b) in the absence of the candidate agent. The amount of complex formed is measured for each sample. An agent modulates the interaction of LXR-alpha and utrophin if the amount of complex formed in the presence of the agent is greater than

(promoting the interaction), or less than (inhibiting the interaction) the amount of complex formed in the absence of the agent. The amount of complex is measured by a binding assay, which shows the formation of the complex, or by using antibodies immunoreactive to the complex.

[0074] Briefly, a binding assay is performed in which immobilized LXR-alpha is used to bind labeled utrophin. The labeled utrophin is contacted with the immobilized LXR-alpha under aqueous conditions that permit specific binding of the two proteins to form a LXR-alpha/utrophin complex in the absence of an added test agent. Particular aqueous conditions may be selected according to conventional methods. Any reaction condition can be used as long as specific binding of LXR-alpha/utrophin occurs in the control reaction. A parallel binding assay is performed in which the test agent is added to the reaction mixture. The amount of labeled utrophin bound to the immobilized LXR-alpha is determined for the reactions in the absence or presence of the test agent. If the amount of bound, labeled utrophin in the presence of the test agent is different than the amount of bound labeled utrophin in the absence of the test agent, the test agent is a modulator of the interaction of LXR-alpha and utrophin.

[0075] Candidate agents for modulating the interaction of each of the protein complexes set forth in Tables 1-10 are screened *in vitro* in a similar manner.

EXAMPLE 16

In vivo Identification of Modulators for Protein-Protein Interactions

[0076] In addition to the *in vitro* method described in Example 15, an *in vivo* assay can also be used to screen for agents which modulate the interaction of LXR-alpha and utrophin. Briefly, a yeast two-hybrid system is used in which the yeast cells express (1) a first fusion protein comprising LXR-alpha or a fragment thereof and a first transcriptional regulatory protein sequence, e.g., GAL4 activation domain, (2) a second fusion protein comprising utrophin or a fragment thereof and a second transcriptional regulatory protein sequence, e.g., GAL4 DNA-binding domain, and (3) a reporter gene, e.g., β-galactosidase, which is transcribed when an intermolecular complex comprising the first fusion protein and the second fusion protein is formed. Parallel reactions are performed in the absence of a test agent as the control and in the presence of the test agent. A functional LXR-alpha/utrophin complex is detected by detecting the amount of reporter gene expressed. If the amount of reporter gene expression in the presence of the test agent is different than the amount of reporter gene expression in the absence of the test agent, the test agent is a modulator of the interaction of LXR-alpha and utrophin.

[0077] Candidate agents for modulating the interaction of each of the protein complexes set forth in Tables 1-10 are screened *in vivo* in a similar manner.

[0078] While the invention has been disclosed in this patent application by reference to the details of preferred embodiments of the invention, it is to be understood that the disclosure is intended in an illustrative rather than in a limiting sense, as it is contemplated that modifications will readily occur to those skilled in the art, within the spirit of the invention and the scope of the appended claims.

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